ABSTRACT

BRCA1 and BRCA2 are the most important breast and ovarian cancer susceptibility genes. Biallelic mutations in BRCA2 can lead to Fanconi anemia and predisposition to cancers, whereas biallelic BRCA1 mutations have not been confirmed, presumably because one wild-type BRCA1 allele is required during embryogenesis. This study describes an individual who was diagnosed with ovarian carcinoma at age 28 and found to have one allele with a deleterious mutation in BRCA1, c.2457delC (p.Asp821Ilefs*25), and a second allele with a variant of unknown significance in BRCA1, c.5207T>C (p.Val1736Ala). Medical records revealed short stature, microcephaly, developmental delay, and significant toxicity from chemotherapy. BRCA1 p.Val1736Ala cosegregated with cancer in multiple families, associated tumors showed loss of wild-type BRCA1, and BRCA1 p.Val1736Ala showed reduced DNA damage localization. These findings represent the first validated example of biallelic deleterious human BRCA1 mutations and have implications for the interpretation of genetic test results.

SIGNIFICANCE: Accurate assessment of genetic testing data for BRCA1 mutations is essential for clinical monitoring and treatment strategies. Here, we report the first validated example of an individual with biallelic BRCA1 mutations, early-onset ovarian cancer, and clinically significant hypersensitivity to chemotherapy.

INTRODUCTION

Hereditary breast and ovarian cancer syndrome is predominantly caused by heterozygous, germline mutation in the BRCA1 or BRCA2 genes (1). Several forms of Fanconi anemia, characterized by bone marrow failure and malignancy, can be a consequence of biallelic mutations in BRCA2 (2) or biallelic mutations in genes encoding BRCA2- and BRCA1-associated proteins PALB2 and BRIP1 (3–7). Despite a frequency of approximately 1.5% in the Ashkenazi Jewish population for BRCA1 and BRCA2 mutations (1,2), the frequency of biallelic mutations in BRCA1 and BRCA2 and their clinical impact remain incompletely understood.

Biallelic Deleterious BRCA1 Mutations in a Woman with Early-Onset Ovarian Cancer

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See related commentary by D’Andrea, p. 376
Figure 1. Pedigrees of families with BRCA1 p.Val1736Ala. A, pedigree of the index family. Circles indicate females and squares indicate males. Slashes indicate death. The proband is indicated by an arrow. Shading in the left lower quadrant indicates ovarian cancer. Shading in the left top quadrant indicates unilateral breast cancer, and in both left and right top quadrants, bilateral breast cancer. Shading in the right bottom quadrant indicates cancer that is not breast or ovarian. Current ages or age at death and age at cancer diagnosis are listed below each individual, as is genetic status if known. B, a second representative pedigree with the BRCA1 p.Val1736Ala alteration. B1, BRCA1; B2, BRCA2; unk, unknown; dx, diagnosis.

the BRCA1 mutations, 185delAG [Human Genome Variation Society (HGVS) c.68_69delAG] and 5382insC (HGVS c.5266dupC), no homozygous or compound heterozygote carriers of these mutations have been reported. Although Brca1 nullizygosity results in embryonic lethality in mice (8), genetically engineered mice harboring biallelic mutations that correspond to human cancer-associated missense mutations within the BRCA1 carboxyl-terminal (BRCT) domain are viable through adulthood and display highly penetrant cancer susceptibility (9). These findings raise the possibility that a partially functional BRCA1 allele in trans with a deleterious truncating mutation (or with a similarly partially functioning mutation) in BRCA1 could be present within the same individual and could contribute to familial cancer susceptibility in humans. Herein, we document the presence of a functionally deleterious BRCA1 BRCT domain missense alteration in trans with a pathogenic BRCA1 alteration in a woman with dysmorphic features and early-onset ovarian carcinoma.

RESULTS

The proband (Fig. 1A, ego 28, arrow) presented at age 28 with stage IV papillary serous ovarian carcinoma. Medical records revealed a history of microcephaly, short stature (adult height of 150 cm), and developmental delay with limited speech at age 4 years. A review of pictures provided by the family showed coarse features with low anterior hairline, macrognathia, a prominent nasal bridge, and small alae nasi. She did not have obvious abnormalities of her thumbs...
Biallelic BRCA1 mutations

Table 1. Loss of heterozygosity from the index family

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Description</th>
<th>Germline BRCA1</th>
<th>Taqman result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2504</td>
<td>Cell line</td>
<td>WT/WT</td>
<td>A/A</td>
<td>No LOH</td>
</tr>
<tr>
<td>S2366</td>
<td>Tumor (breast)</td>
<td>WT/WT</td>
<td>A/A</td>
<td>No LOH</td>
</tr>
<tr>
<td>Ego 1</td>
<td>Lymphoblasts</td>
<td>WT/p.Val1736Ala</td>
<td>A/G</td>
<td>No LOH</td>
</tr>
<tr>
<td>Ego 10</td>
<td>Lymphoblasts</td>
<td>WT/p.Val1736Ala</td>
<td>A/G</td>
<td>No LOH</td>
</tr>
<tr>
<td>Ego 1</td>
<td>Tumor (Breast)</td>
<td>WT/p.Val1736Ala</td>
<td>G/G</td>
<td>LOH at WT</td>
</tr>
<tr>
<td>Ego 9</td>
<td>Tumor (Ovarian)</td>
<td>WT/p.Val1736Ala</td>
<td>G/G</td>
<td>LOH at WT</td>
</tr>
<tr>
<td>Ego 28</td>
<td>Tumor (Ovarian)</td>
<td>p.2576delC/p.Val1736Ala</td>
<td>A/G</td>
<td>No LOH</td>
</tr>
</tbody>
</table>

NOTE: Ovarian/primary peritoneal cancer tumor blocks from Family A egos 1 and 9 showed that LOH had occurred at the wild-type BRCA1 allele with retention of the p.Val1736Ala allele. ‘A’ at position 5207 represents the wild-type allele and ‘G’ at position 5207 represents the Val1736Ala allele. Conversely, in ego 28, who carried germline BRCA1 2576delC and p.Val1736Ala alterations in trans, the ovarian tumor did not display LOH at either allele.

Abbreviation: LOH, loss of heterozygosity; WT, wild type.

and had a normal complete blood count at the time of her cancer diagnosis. Neither ataxia nor telangiectasias were documented. This individual was found to have a known deleterious mutation in BRCA1 reported as 2576delC (HGVS c.2457delC, p.Asp821Ilefs*25) and a variant of unknown significance (VUS) in BRCA1 (HGVS c.5207T>C) p.Val1736Ala, as well as a VUS in BRCA2 (HGVS, c.3971G>C, p.Arg324Thr).

Treatment with carboplatin [target area under the concentration versus time curve in mg/mL] resulted in significant toxicity requiring neutrophil count (ANC) nadir of 160/mm^3, as well as grade 2 thrombocytopenia (nadir of 3,000/mm^3), for which she received red blood cell and platelet transfusions. She also developed anemia (hemoglobin nadir of 7.8 g/dL) and grade 4 thrombocytopenia (nadir of 3,000/mm^3), for which she received red blood cell and platelet transfusions. She also developed grade 2 nausea, diarrhea, and mucositis. As a result of the excess toxicity, carboplatin and paclitaxel were discontinued after 2 cycles. She received no further therapy and died 6 months after her diagnosis. Extreme sensitivity to the interstrand crosslinking agent carboplatin is not typically observed in heterozygous BRCA1 mutation carriers (10–12), but it is seen in biallelic BRCA1-mutant cells and mice, suggesting that both BRCA1 alleles were compromised for DNA repair function.

The mother of ego 28 was diagnosed with ovarian cancer at age 53 and died at 55. A maternal great aunt (Fig. 1A, Family A, ego 1) was diagnosed with breast and ovarian cancers at ages 59 and 69, respectively, and a contralateral breast cancer at age 76. A second maternal great aunt (Fig. 1A, Family A, ego 10) was diagnosed with breast cancer at age 67 and died at 68. Notably, both carried the BRCA1 p.Val1736Ala variant VUS but not the known pathogenic mutation BRCA1 2576delC (HGVS c.2457delC).

Additional genetic testing in the family revealed that the brother of the proband (ego 27) carries the BRCA1 c.2457delC mutation and the paternal lineage also had multiple cases of early-onset breast cancer. To investigate this variant further, we were able to obtain pedigrees on 11 additional families with the BRCA1 p.Val1736Ala VUS. Nine of these pedigrees that had additional genotyping of family members were used to assess cosegregation using methods described by Thompson and colleagues (ref. 13; a representative pedigree is shown in Fig. 1B). The combined OR in favor of p.Val1736Ala being pathogenic was 234:1, assuming the age-specific penetrance estimated in Antoniou and colleagues (14). Loss of heterozygosity (LOH) analysis was conducted on genomic DNA extracted from BRCA1 p.Val1736Ala mutation-positive tumors using a custom designed TaqMan assay (Table 1).

The BRCA1 BRCT residue Val1736 is conserved across 18 different vertebrate species (Fig. 2A). In contrast with other BRCT residues that exhibit cancer-associated point mutations, structural models predict that Val1736 does not make direct contact with phosphopeptide ligands (Fig. 2B). Rather, Val1736 resides in a hydrophobic pocket, which may affect the stability of residues Pro1749 and Cys1697, both of which are required for BRCT function in DNA repair and tumor suppression. Transfection of a DNA double-strand break (DSB) reporter cell line (ref. 15; Supplementary Fig. S2) with an epitope-tagged carboxy-terminal region of BRCA1 revealed that a wild-type (WT) BRCT fragment was observed at more than 80% of DSBs, whereas the fragment containing p.Val1736Ala was reduced to less than 40% (P = 0.0029), intermediate to the WT protein and known BRCT-mutant p.Pro1749Arg, which was present at less than 20% of DSBs (P = 0.0017; Fig. 2C and D). Similarly, coimmunoprecipitation experiments with the same epitope-tagged BRCA1 fragments showed significantly diminished interaction between p.Val1736Ala and RAP80, a BRCA1 BRCT-interacting protein, in comparison with WT BRCT-containing fragments (Fig. 2E). Consistent with these results, overexpression of the WT BRCT fragment acted as a dominant-negative allele by reducing...
Figure 2. Analysis of the BRCA1 p.Val1736Ala mutation. A, partial sequence alignment of a BRCA1 BRCT domain from different species showing that BRCA1 V1736 and P1749 residues (highlighted in red) are completely conserved across all vertebrate species. Numbers on top of the alignment indicate amino acid positions of the human BRCA1 protein. Conservation below describes sequence conservation (*, identical; ≥ 80% conservation; ., ≥ 60% conservation).

B, modeling [based on protein data bank (pdb) code 1t15] of the interaction between the BRCA1 BRCT domains and a peptide of BACH1. BRCA1 is gray, with disease-causing mutants of the conserved residues in red. The BACH1 peptide is purple.

C, WT BRCA1 (shown as a green focus) but not the p.Val1736Ala or p.Pro1749Arg mutant efficiently colocalized with mcherryLacIFokI fusion endonuclease-induced DNA DSBs.

D, percentage of cells with BRCA1 (WT or mutant) colocalizing to FokI was quantified. At least 100 cells were assessed for each data point (n > 100). Measurements were obtained in triplicate and reported as means of 3 replicates. P values were calculated using Student t test; P < 0.05 for all comparisons. Error bars, SEM.

E, Coimmunoprecipitation of epitope-tagged BRCA1 (Myc-BRCA1) WT or mutants from 293T cells at room temperature for 2 hours followed by immunoblot for RAP80.

IR-induced RAD51 foci formation and homology-directed DSB repair by a significantly greater extent than overexpression of BRCA1 fragments containing either the BRCT domain mutations p.Pro1749Arg or p.Val1736Ala (Supplementary Fig. S3).

We are aware of only one previous report of biallelic deleterious mutations in BRCA1 in humans. In this report, a Scottish woman was found to be homozygous for BRCA1 c.2681_2682delAA (HGVS c.2681_2682delAA, p.Lys894ThrfsX8; ref. 16). This individual was diagnosed with breast cancer at age 32 and subsequently developed a contralateral breast cancer. Homozygosity for this mutation was plausible particularly because it is a founder mutation in the studied population (17). Nevertheless, this report has long been questioned because potential primer bias in PCR-based genotyping could have led to preferential amplification of the putative mutant allele and hence masking of true heterozygosity (18). Because of the importance of this single report for the interpretation of our own results, we resequenced peripheral blood lymphocyte DNA from the reported biallelic carrier and found that only one BRCA1 allele harbored the designated mutation c.2681_2682delAA, whereas the other allele was found to be WT at this position (Fig. 3A).
Biallelic BRCA1 mutations

DISCUSSION

Here, we report the first individual with validated biallelic mutations in BRCA1. Compelling evidence is presented that BRCA1 p.Val1736Ala is both pathogenic and can support viability through adulthood in trans to a deleterious mutation in exon 11 of BRCA1 (BRCA1 2576delC). BRCA1 p.Val1736Ala diminishes protein–protein interaction with RAP80 and localization to DSBs and imparts cancer susceptibility independent of other BRCA1 or BRCA2 alterations. LOH analysis was also consistent with pathogenicity. Loss of the WT allele occurred in both tumors that carried the p.Val1736Ala VUS in trans to WT BRCA1; however, LOH did not occur in the ovarian cancer of the proband (ego 28), which was compound heterozygous for p.Val1736Ala and 2576delC, indicative of a scenario in which selective pressure did not exist to delete either pathogenic allele.

Several features of the index patient were uncharacteristic for monoallelic BRCA1 mutation carriers. In addition to the aforementioned developmental delay, microcephaly, and short stature, ovarian cancer was diagnosed earlier than the age of 30, which is unusual for BRCA1 mutation carriers (19). The patient also had extreme sensitivity to the interstrand crosslinking agent carboplatin, a characteristic not typically displayed in BRCA1 mutation carriers. In addition to the biologic implications, the findings in this study have importance to the interpretation of genetic variants. VUSs are a common finding in genetic testing for inherited cancer syndromes and pose challenges in counseling and management (27). Cooccurrence of a VUS in trans with a known deleterious BRCA1 mutation is considered a strong indication that the VUS is not clinically important (28). Our findings suggest that the presence of a BRCA1 VUS in trans with an established deleterious BRCA1 mutation should not be considered as definitive evidence against pathogenicity. This work also highlights the importance of examining multiple distinct lines of evidence when interpreting a VUS, including clinical phenotype. This lesson is particularly pertinent in the era of massively parallel DNA sequencing, as a large number of VUSs will be identified with the use of this methodology and caution will be needed in interpreting these results for clinical use.

METHODS

LOH Analysis

DNA was extracted from either cell lines or tumors following microdissection of cancer tissue to over 70% tumor (Supplementary Fig. S3). LOH was assessed by the University of Pennsylvania Genomics Facility using a custom-designed TaqMan assay to distinguish a single nucleotide alteration at nucleotide position 5207, codon 1736 from the WT allele (Table 1 and Supplementary Fig. S1).

BRCA1 c.2681_2682delAA Resequencing

Lymphocyte DNA from the patient was amplified by PCR using the primers F1: 5′-AACCACACCTCGGGAACACAG-3′ and R2:
5′-TGATGGGAAAGTGGTGTTG-3′ and the QIAGEN HotStar Plus PCR system according to the manufacturer’s instructions. PCR products were sequenced by Sanger sequencing using the 3730xl DNA Analyzer technology (Applied Biosystems), and the traces were visualized using Chromas software (Technelysium).

**Immunofluorescence**

Immunofluorescence was conducted in the DSB reporter cells as described previously (15). No additional authentication on cell lines was conducted. All analyses were carried out on unmodified images that were captured with a QImaging RETIGA-SRV camera connected to a Nikon Eclipse 80i microscope.

**Disclosure of Potential Conflicts of Interest**

D.E. Goldgar has an ownership interest (including patents) and royalties from BRCA1/2 gene patents. No potential conflicts of interest were disclosed by the other authors.

**Authors’ Contributions**

Conception and design: S.M. Domchek, K.L. Nathanson, W. Foulkes, R.A. Greenberg

Development of methodology: S.M. Domchek, R.A. Greenberg

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.M. Domchek, J. Tang, J. Stopfer, D.R. Lilli, N. Hamel, M. Tischkowitz, A. Yonker, F.J. Couch, H.R. Davidson, K.L. Nathanson, W. Foulkes, R.A. Greenberg


Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S.M. Domchek, J. Stopfer, K.L. Nathanson, R.A. Greenberg

Study supervision: S.M. Domchek, R.A. Greenberg

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